

**REMARKS ON THE DIVERSITY AND ECHOLOCATION CALLS OF
HIPPOSIDERID BATS (Chiroptera: Hipposideridae) IN CUC PHUONG
NATIONAL PARK, NORTHEASTERN VIETNAM**

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ABSTRACT

Cuc Phuong is the oldest and most well-known National Park in Vietnam. With its large primary forest area and karst cave systems, it is assumed to be an ideal home to a highly diverse bat fauna. However, species diversity and echolocation of bats in this park are poorly characterized. Between April 2021 and April 2022, three bat surveys were conducted in the park with special attention on the species of the family Hipposideridae. The bat specimens collected previously from the park were also examined. According to past records, hipposiderids in the park comprised at least seven species: *Aselliscus stoliczkanus*, *Coelops frithii*, *Hipposideros alongensis*, *Hipposideros armiger*, *Hipposideros gentilis*, *Hipposideros lylei* and *Hipposideros poutensis*. In this series of field surveys, six of these seven species were captured while *Coelops frithii* was only confirmed from specimen examination. Each species is clearly distinguishable by their morphology and echolocation calls. This study provides the first comparison of echolocation calls of all seven species which will be useful for potential acoustic identification and monitoring bats in the park and surrounding areas in future.

Keywords: Echolocation, ecology, identification, monitoring, morphology.

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INTRODUCTION

Cuc Phuong was first established as a protected area, namely “Cuc Phuong Forest Enterprise”, in 1962 and upgraded to a National Park in 1966 (BIIP, 2001). It is located in northeastern Vietnam (20°14′–20°24′N; 105°29′–105°44′E) and covers an area of 22,200 ha with a wide range of natural ecosystems including caves and primary forests which are suitable for many bat species (BIIP, 2001). However, bats in Cuc Phuong National Park are poorly known and rarely documented. Le Hien Hao (1971) was the first who published the record of bats from the park. Subsequently, based on the literature review, Dang Huy Huynh et al. (1994) included the records of ten bat species from the park in the first version of the “Checklist of Mammals in Vietnam”. Subsequently, bat surveys were carried out several times in the park between 1997 and 2001 (Bates et al., 1997; Bates et al., 1999; Hendrichsen et al., 2001). Specimen examinations and literature reviews for those surveys revealed that eight hipposiderid species were recorded by those surveys: *Aselliscus stoliczkanus*, *Coelops frithii*, *Hipposideros armiger*, *Hipposideros bicolor*, *Hipposideros larvatus*, *Hipposideros lylei*, *Hipposideros pomona* and *Hipposideros turpis* (Hendrichsen et al., 2001; Le Vu Khoi et al., 2001; Borrisenko & Kruskop, 2003; Dang Ngoc Can et al., 2008; Thong et al., 2012; Kruskop, 2013). However, echolocation calls of the bats from the park were almost undocumented in the past publications, although they are useful for acoustic identification, monitoring, and other research purposes (Schnitzler & Kalko, 1998; Furey et al., 2009; Schnitzler & Denzinger, 2011; Denzinger & Schnitzler, 2013; Denzinger et al., 2016). The only publication containing a call parameter (the values of the constant-frequency modulated component) of *Hipposideros alongensis* was by Thong et al. (2012) which was subsequently cited by Kruskop (2013). To fill in this knowledge gap on echolocation research in the park, three field surveys were conducted between April 2021 and April

2022, and museum specimens were examined as well. This study provides confirmation of hipposiderid bat species composition following the current taxonomy (Simmons & Cirranello, 2023). Also, the comparison of echolocation data of 8 hipposiderid bat species provide a basis for future acoustic identification and potential monitoring or ecological research in the park and the surrounding areas.

MATERIALS AND METHODS

Bat capture and identification

Three field surveys were conducted at the caves and in the forest habitats of Cuc Phuong National Park in 2021 (April 12–24; June 2–14) and 2022 (April 2–11). Mist nets of 6.0–9.0 m (height) × 9.0–12.0 m (length), mesh size 16 × 16 mm and four-bank harp traps (Francis, 1989) were used to capture bats. The traps were set up in front of the caves or under the canopy of plantations and natural vegetation, while the nets were raised to the level of forest canopies. Bats were captured and handled according to the guidelines of the American Society of Mammalogists (Sikes & Gannon, 2011; Sikes et al., 2016). Netted or trapped bats were carefully removed from the nets or traps and their reproductive status and age were examined following Racey (2009) and Brunet-Rossini & Wilkinson (2009). Juveniles were released and excluded from the analyses of identification to reduce the influence of age variation. Four morphological measurements (forearm length - FA, Ear height - EH, Ear width - EW, and noseleaf width - NW) of every captured adult individual were taken using callipers, then identified through a comparison with the data of the species in the past publications (Bates et al., 1997; Bates et al., 1999; Hendrichsen et al., 2001; Borrisenko & Kruskop, 2003; Thong et al., 2012; Kruskop, 2013; Wilson & Mittermeier, 2019; Yuzefovich et al., 2021; Yuzefovich et al., 2022). A total of 238 bat individuals and 61 museum specimens were captured and examined, respectively. All the individuals were released at the capture sites after taking the selected measurements and

photographs for morphological identification. Wing punches from representative individuals of each species form were collected for genetic data to confirm the taxonomy. Because all captured hipposiderid bats exhibited well the diagnostic characteristics of respective species, they were identified morphologically in the field without difficulty. Therefore, the methods and relevant information for genetic data are not included in this paper. The 61 museum specimens are preserved in the Department of Zoological Museum, Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology, Ha Noi, Vietnam.

Echolocation recordings and analyses

All known hipposiderid bats in the park are cave-dwelling species. Therefore, echolocation calls were recorded using the PCTape system (480 kHz, 16 bit) at three different situations: emerging from caves, while bats were handheld, flying inside a flight tent [5 m width, 5 m length, 3 m height]. Batman software, which displays color sonograms of the detected echolocation signals in real-time, was used to obtain high-quality sound sequences. Selected sound sequences were analysed using the Selena software. PCTape, Batman and Selena are all custom-made programs (version May 2008) by the University of Tübingen, Germany. Hipposiderid bats use multiharmonic echolocation calls with the highest energy in the second harmonic (Thong et al., 2012). Each entire harmonic comprises two components: constant-frequency followed by a downward frequency modulated sweep. Therefore, selected recordings were displayed as sonograms with an FFT (Fast Fourier Transformation) of 512, Hann-window, and zero-padding to measure the constant frequency of the second harmonic (CF2).

RESULTS

Acoustic and morphological identification

Six hipposiderid bat species were captured over the three field surveys: *A. stoliczkanus*,

H. alongensis, *H. armiger*, *H. gentilis*, *H. lylei* and *H. poutensis*. *C. frithii* was not captured during the surveys but its presence was confirmed based on the examination of a single museum specimen (IEBR-T.030908.1) which is preserved at the Department of Zoological Museum, Institute of Ecology and Biological Resources, Ha Noi, Vietnam. The six captured species were distinguishable from each other in both morphological and acoustic features and also from *C. frithii* in morphology (Figs. 1, 2; Table 1).

A. stoliczkanus is a small and particularly sensitive species of the family Hipposideridae. While its body size was similar to that of *H. gentilis*, its noseleaf structure was different from that of all other hipposiderid species found in the park and can be easily identified (Fig. 1A). The forearm lengths of 41 captured individuals of *A. stoliczkanus* were in a range of 41.5–43.8 mm. The captured individuals emitted their calls with the CF2 values in a range of 120.1–123.1 kHz, which is lower than that of *H. gentilis* (Table 1). The CF2 value ranges of *A. stoliczkanus* and the following species exhibit the inter-individual variations.

H. armiger is the largest species in the genus *Hipposideros* found in the park with an average forearm length of 91.9 mm (85.6–96.4 mm). It was distinct from all other species by body size and external characteristics. Its noseleaf had four supplementary leaflets (Fig. 1C). Three inner leaflets were well developed while the outermost one was less developed. *H. armiger* used an echolocation call structure typical to hipposiderid bats. The frequency value range for CF2 of the captured individuals is 65.9–68.6 kHz (Table 1).

The Vietnamese endemic, *H. alongensis* was also found in the park. It is similar to *H. poutensis* in noseleaf structure (Fig. 1B) but clearly distinct in body size and echolocation frequency (Table 1). The forearm lengths of 38 captured individuals ranged from 69.0–72.5 mm. They emitted their echolocation calls with CF2 values in the range of 73.1–75.1 kHz (Table 1).



Figure 1. Frontolateral view of *Aselliscus stoliczkanus* (A), *Hipposideros alongensis* (B), *Hipposideros armiger* (C), *Hipposideros gentilis* (D), *Hipposideros lylei* (E) and *Hipposideros poutensis* (F) from Cuc Phuong National Park

The smallest species found in the park is *H. gentilis* with an average forearm length of 42.0 mm (40.3–43.0 mm). It was distinguishable from the others based on the combined traits of body size, noseleaf structure, and echolocation call frequency. Its noseleaf structure is quite simple without supplementary leaflets (Fig. 1D). As a hipposiderid species, each echolocation signal of this species also comprised a short constant-frequency component followed by a frequency modulated sweep. The captured individuals emitted their calls with the CF2 values in a range of 126.3–129.0 kHz (Table 1).

H. lylei is a medium-sized species of *Hipposideros* and is clearly distinguishable from the six others by its noseleaf structure

with a dominant “shield” behind the posterior leaf (Fig. 1E). The forearm lengths of 32 captured individuals were in the range of 75.4–78.8 mm. The echolocation call structure is similar to that of *H. alongensis* but distinguishable given the lower CF2 values for *H. lylei*. The captured individuals emitted their echolocation calls with the CF2 values in the range of 70.8–71.5 kHz (Table 1).

H. poutensis is a medium-sized hipposiderid species of *Hipposideros* and can be distinguished from the others by its body size, supplementary leaflets and CF2 values. The forearm lengths of 56 captured individuals were in the range of 60.7–63.8 mm. Its noseleaf had three supplementary leaflets which were all more or

less equal in size (Fig. 1F). The echolocation call of this species is similar to that of *H. armiger* in signal structure but had a

distinct range in the CF2 values. The CF2 values of the captured individuals were in a range of 93.6-94.7 kHz (Table 1).

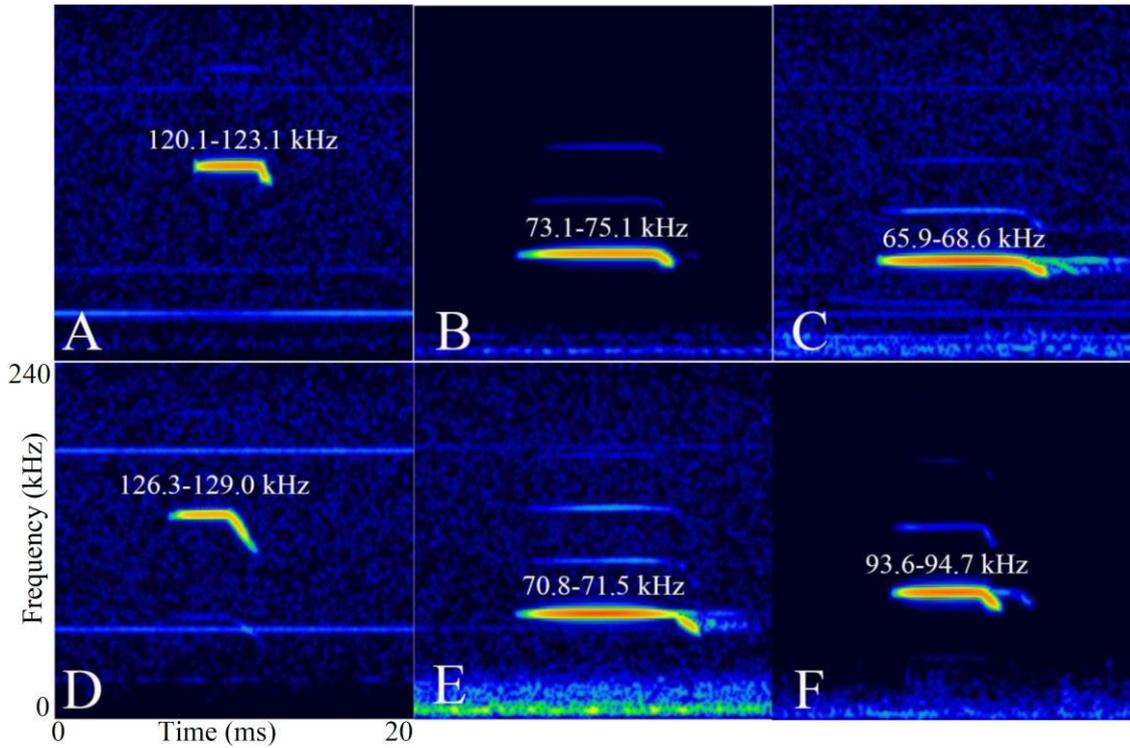


Figure 2. Echolocation calls of *Aselliscus stoliczkanus* (A), *Hipposideros alongensis* (B), *Hipposideros armiger* (C), *Hipposideros gentilis* (D), *Hipposideros lylei* (E) and *Hipposideros poutensis* (F) from Cuc Phuong National Park

Table 1. Morphological measurements and CF2 values of the captured bats in Cuc Phuong National Park. Data are given as mean \pm standard deviation and minimum - maximum

Species name	n	FA	EH	EW	NW	CF2
<i>Aselliscus stoliczkanus</i>	41	42.7 \pm 0.6	10.2 \pm 0.5	7.4 \pm 0.7	5.5 \pm 0.3	121.5 \pm 0.9
		41.5–43.8	9.4–11.2	6.1–8.9	5.1–6.1	120.1–123.1
<i>Hipposideros alongensis</i>	38	70.9 \pm 1.3	27.2 \pm 1.3	22.5 \pm 0.7	10.2 \pm 0.4	74.1 \pm 0.8
		69.0–72.5	25.0–28.0	21.5–23.0	10.0–11.0	73.1–75.1
<i>Hipposideros armiger</i>	38	91.9 \pm 3.0	31.8 \pm 1.1	27.5 \pm 2.1	10.0 \pm 1.4	67.2 \pm 1.0
		85.6–96.4	29.5–33.0	23.0–29.5	7.5–12.0	65.9–68.6
<i>Hipposideros gentilis</i>	33	42.0 \pm 0.8	21.9 \pm 0.9	17.2 \pm 0.5	4.7 \pm 0.2	127.0 \pm 0.8
		40.3–43.0	20.9–23.7	16.4–17.8	4.4–5.0	126.3–129.0
<i>Hipposideros lylei</i>	32	77.2 \pm 1.7	25.5 \pm 3.9	23.0 \pm 0.5	10.5 \pm 1.3	71.2 \pm 0.4
		75.4–78.8	21.0–28.0	22.5–23.5	9.5–12.0	70.8–71.5
<i>Hipposideros poutensis</i>	56	62.6 \pm 1.5	21.0 \pm 0.4	19.4 \pm 0.3	7.0 \pm 0	94.1 \pm 0.5
		60.7–63.8	20.5–21.5	19.0–19.5	7.0–7.0	93.6–94.7

The morphological features of the captured individuals were congruent with those of the previously collected specimens except for the slight variation of the noseleaf width of several specimens (Tables 1 & 2). Echolocation calls of individuals which were from museum collections did not provide any recordings from field surveys to allow comparison with the bats caught in this study. Based on their agreement

in morphological features between the captured bats and museum specimens though, the CF2 values of those individuals are likely to be identical with the CF2 values of captured individual of each respective species. The CF2 values of each species from three recording scenarios (emerging from caves, while bats were handheld, flying inside a flight tent) overlapped.

Table 2. External measurements of the examined specimens housed in IEBR

Species name	n	FA	EH	EW	NW
<i>Coelops frithii</i>	1	36.69	12.5	15.0	5.5
<i>Aselliscus stoliczkanus</i>	6	42.5 ± 0.5	10.3 ± 0.2	7.5 ± 0.4	5.3 ± 0.1
		42.3–43.8	9.4–11.0	6.2–8.5	5.3–6.0
<i>Hipposideros alongensis</i>	11	70.9 ± 0.8	26.8 ± 0.5	22.3 ± 0.6	10.3 ± 0.2
		70.1–71.3	26.1–27.9	21.8–22.9	10.0–10.8
<i>Hipposideros armiger</i>	8	93.2 ± 1.5	32.0 ± 0.6	28.1 ± 0.9	10.5 ± 1.2
		88.5–95.3	30.8–32.5	24.5–28.3	7.6–11.5
<i>Hipposideros gentilis</i>	9	41.3 ± 0.5	21.5 ± 0.3	16.9 ± 0.3	4.8 ± 0.1
		40.9–42.6	20.9–22.8	16.6–17.2	4.5–4.8
<i>Hipposideros poutensis</i>	26	62.3 ± 0.9	21.0 ± 0.2	19.3 ± 0.2	7.0 ± 0.3
		61.8–63.2	20.8–21.3	19.0–19.4	6.8–7.1

DISCUSSION

Among the seven confirmed hipposiderid species from the park, the taxonomic status of three species (*H. alongensis*, *H. gentilis*, *H. poutensis*) was updated following the current taxonomy (Simmons & Cirranello, 2023). Previous publications included records of *H. bicolor*, *H. pomona*, *H. turpis* and *H. larvatus* (Hendrichsen et al., 2001; Le Vu Khoi et al., 2001; Borisenko & Kruskop, 2003; Dang Ngoc Can et al., 2008; Kruskop, 2013). However, Kruskop (2013) indicated that “records of *H. bicolor* from Vietnam should be treated as mistakes, based on misidentified *H. pomona*” while Yuzefovich et al. (2021) suggested that the population of “*H. pomona*” from northern Vietnam including the park “make doubtful the subspecies status of *H. gentilis sinensis*”. Therefore, the records of *H. bicolor* from the park in previous publications were evidentially due to misidentification of specimens belonging to *H. gentilis*. The present study confirmed the occurrence of

H. gentilis in the park (Tables 1 & 2). Likewise, Thong et al. (2012) and Yuzefovich et al. (2022) also indicated that the previously published records of *H. turpis* and *H. larvatus* from northern Vietnam including the park were due to the misidentification of specimens belonging to *H. alongensis* and *H. poutensis*, respectively. The populations of *H. alongensis* in Cuc Phuong National Park and other karst areas in the mainland of Vietnam belong to the subspecies, *H. alongensis sungi*, which is distinct from another subspecies in island ecosystems within the Cat Ba and Ha Long Bay archipelagoes, *H. alongensis alongensis* (Thong et al., 2012; Kruskop, 2013; Wilson & Mittermeier, 2019).

To date, the genus *Aselliscus* is comprised of two species in Vietnam: *A. stoliczkanus* and *A. dongbacanus* (Wilson & Mittermeier, 2019). These two species differ in size and shape of their upper canines, as well as in their genetic data (Wilson & Mittermeier, 2019). The upper canines of the museum specimens examined and the captured

individuals from the park exhibited the diagnostic features of *A. stoliczkanus*. Additionally, genetic data of the captured individuals from the park also supported the morphological identification of this species (Sofia Hayden, pers. comm.).

C. frithii is listed as a globally Near Threatened by the current IUCN Red List of Threatened Species (Huang et al., 2019). It was not captured in the recent field surveys. It is a small hipposiderid species with round and thin ears (Fig. 3). Its echolocation calls share distinct from those of the six remaining species and other hipposiderid species in both signal structure and CF2 values (Vu Dinh Thong, 2021; Fig. 3). Generally, *C. frithii* is distinct from all other hipposiderid bat species of the park as well as Vietnam nationwide in both morphology and echolocation (Wilson & Mittermeier, 2019; Vu Dinh Thong, 2021; Fig. 3). Each entire echolocation harmonic of this species comprises a short constant-frequency or occasionally appears as an upward frequency modulated (iFM) followed by a large

bandwidth downward terminal frequency modulated (tFM) component (Fig. 3). The CF2 value of this species is around 220 kHz which is also distinctly high in comparison with that of all other hipposiderid bat species in Vietnam and surrounding countries (Vu Dinh Thong, 2021).

These species are all distinguishable by both their morphology and acoustic features. Given our robust sampling of individuals from the park, the recorded CF2 values can be regarded as representative echolocation calls for acoustic identification and monitoring of hipposiderid bat species in the park. However, echolocation call frequency can vary between sexes, ages, geography, habitats and other factors (Jones & Kokurewics, 1998; Jiang et al., 2010). Thus, the use of these data as a reference for other sites should be approached with caution. Further studies on echolocation calls in the park comparison with the results from other localities are required for a comprehensive understanding of hipposiderid and other bat species through their distributional ranges.

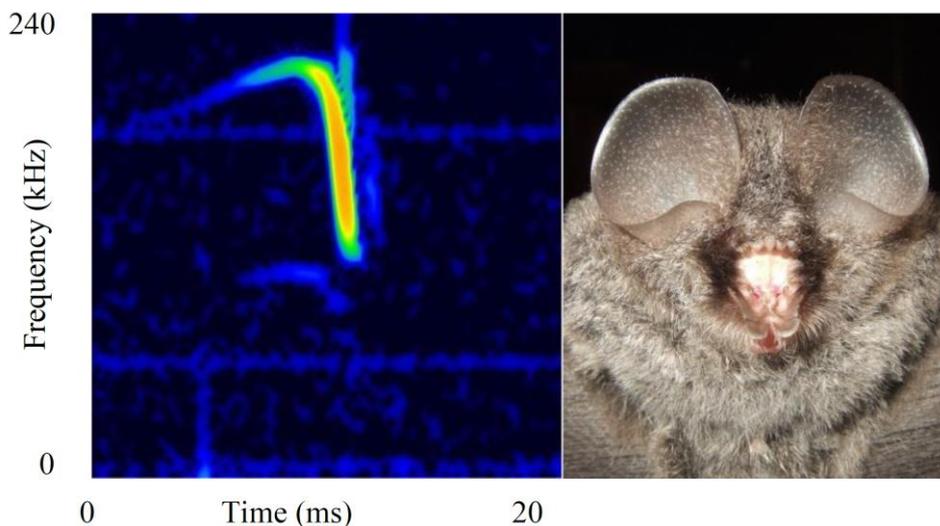


Figure 3. Echolocation call (left) and frontal view (right) of *Coelops frithii* previously recorded from Cuc Phuong National Park (adapted from Vu Dinh Thong, 2021)

CONCLUSION

Cuc Phuong National Park is home to at least seven hipposiderid bat species belonging

to three genera: *A. stoliczkanus*, *C. frithii*, *H. alongensis*, *H. armiger*, *H. gentilis*, *H. lylei* and *H. poutensis*. These species are clearly distinguishable from each other in both

morphology and echolocation. *C. frithii* was the rarest species and was not recorded during the three field surveys over two years. The first comparative echolocation data from the present study can be used for acoustic identification, potential monitoring and ecological studies within the park and surrounding areas in the future.

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